# PROTOPLASMIC VISCOSITY CHANGES DURING MITOSIS IN THE EGG OF CHAETOPTERUS

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In spite of the enormous effort that has been spent in order to discover the basic cause of cancer, a disease primarily due to the initiation of cell division in cells which normally do not divide, there has been but little advance in the past twenty years in our understanding of the basic physiology of cell division. Of the three main theories which have been proposed to account for the initiation of cell division, only one survives and that has had but little test. The idea that cell division is caused by an increase in cell permeability can scarcely be held at the present time, for in marine egg cells the calcium ion is a potent agent for promoting mitosis (See Pasteels, 1941; Hollingsworth, 1941) and the calcium ion is well known for its effect in decreasing rather than increasing cell permeability. Secondly, at the present time it can hardly be maintained that an increase in respiration is the primary cause of initiation of cell division. When certain types of cells are incited to divide, the respiration does increase, but other types of cells show no such effect, and in still other cells the respiration decreases (Whitaker, 1931a, b, c; 1933a, b; compare also Holter and Zeuthen, 1944). Nor, on the basis of present evidence, can it be held that a particular respiratory system is involved; at any rate the attempt to argue that in the sea-urchin egg the cytochrome oxidase system is activated when the cell is incited to divide is rather an expression of wishful thinking than of careful experimentation (See Robbie, 1946). We are left then with the third of the three major theories, the view that the primary impetus to cell division is a mitotic gelation akin to the gelation which occurs generally in cells when they respond to stimulation. This colloidal theory of cell division is discussed at some length in the second edition of Heilbrunn's Outline of General Physiology (1943, see Chapt. 42).

One of the reasons for believing in the colloidal theory is that in those cells in which viscosity studies have been made, the appearance of the mitotic spindle appears to be preceded by a very definite gelation of the cytoplasm. Actually, however, only a few types of cells have been studied, and if this point is to be firmly established, we should have additional information for other types of cells.

We were led to a study of the egg of the annelid Chaetopterus pergamentaceus Cuvier, because this worm is found in suitable numbers at Woods Hole and can be obtained for study, and also because the egg represents a type similar to Cumingia in that fertilization occurs at the time of the first maturation spindle. Unfortunately, at the present time Cumingia is very rare at Woods Hole and therefore can not be used for experimental work.

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Chaetopterus eggs can be obtained throughout the summer (Compare Mead, 1898). One female worm provides enough eggs for several experiments. When the eggs are shed into the sea water, they are in the germinal vesicle stage, but as soon as they enter the sea water, maturation begins and proceeds until the metaphase stage of the first maturation division is reached. Then, if the egg is fertilized, the maturation divisions continue and cleavage follows.

Obviously, the Chaetopterus egg is convenient for study. We plan to use it in various types of experimental work. We were interested therefore in knowing the normal cycle of viscosity change and how this cycle was related to the mitotic phenomena occurring between the time of fertilization and the first cleavage. Strangely enough, in spite of the great amount of cytological work on mitosis, there is no complete minute by minute time record of what can be seen in fixed and sectioned material during the progressive stages of mitosis in marine eggs.

## Methods

The sexes of Chaetopterus can be determined from the color of the gametes contained in the transparent parapodia. The eggs are yellow and the sperm are white. A few posterior segments of a worm were cut off and placed in a small stender dish containing about 20 ml. of sea water. The parapodia of these excised segments were cut open and as a result the eggs or the sperm, as the case might be, exuded into the sea water. Eggs were filtered through cheesecloth to remove the excess jelly and extraneous tissue and then were washed by decantation. The eggs are usually so abundant that only a few segments of the worm provide enough eggs for a single experiment. A sperm suspension was prepared by removing the parapodia and segments from the stender dish and then adding to the original 20 ml. another 10 ml. of sea water. Two or three drops of this suspension were used to fertilize one batch of eggs. A worm does not die following the removal of several segments and indeed the same worm may be used several times.

In all of our work, the eggs of only one female were used in any given series of experiments. The eggs were kept in a water bath maintained at a constant temperature of 21° C. In some experiments, the temperature varied slightly, but ordinarily the variation was not greater than two or three tenths of a degree. As soon as the eggs began to show indications of cleavage, counts were made as rapidly as possible in order to determine with reasonable precision the exact time at which 50 per cent of the eggs had divided. In these counts, all eggs in which a cleavage plane had begun to travel across the egg were regarded as cleaved. Actually, the passage of the cleavage plane through the egg takes an appreciable time, perhaps a minute or more, so that the cleavage times we recorded are somewhat less than the times would be if we considered as cleavage time the time at which the egg is completely divided. In making rapid counts on living eggs, it would scarcely be possible to use as a criterion of cleavage the complete division of the egg. At a temperature of 21° C. we found the average cleavage time to be 56 minutes.

Protoplasmic viscosity tests were made with an Emerson hand centrifuge. The handle of the centrifuge was turned once every two seconds. This represents a speed of 85 revolutions per second. The radius of turn was 8 cm. The centrifugal force was calculated to be 2325 times gravity. A few preliminary tests of protoplasmic viscosity during mitosis showed that the viscosity changes in the

dividing Chaetopterus egg are not as pronounced as they are in the egg of Cumingia or in the egg of Arbacia. If tests are to be made at frequent intervals, observations must be made rapidly. This can introduce uncertainty. We were worried over the possibility that subjective impressions might creep in. Accordingly, we decided on the following procedure. For any given test, the centrifuge was turned by Mrs. Jean Wilson. As soon as the turning was completed, she passed the centrifuge tube to one of us as quickly as possible. The eggs were then removed from the tube to two microscope slides. Each of us had a microscope, and we observed the centrifuged eggs independently. In this way, we were able to make tests at one minute intervals and although the observations were necessarily very rapid, when we compared our results at the end of a series of tests, we found remarkably good agreement.

When a Chaetopterus egg is centrifuged, lighter (presumably fat) granules move to the centripetal pole, and heavier yolk granules move to the centrifugal pole. There is a cortical layer of granules which does not move at all. Details of the appearance of centrifuged eggs are given by Lillie (1906). When we observed the centrifuged eggs, we recorded everything that we could see. If the viscosity is relatively low, the yolk granules move more readily through the egg; the fat granules also move more readily. It is possible to observe a shift of the yolk granules before any movement of the fat granules can be noted. One can follow viscosity change either by considering the movement of heavy or light granules. In order to obtain definite quantitative values for viscosity, we chose as an endpoint the appearance of a definite accumulation of fat granules at the centripetal pole of the egg. The number of seconds required to give this accumulation was used in plotting a viscosity curve.

The eggs of Chaetopterus are not very transparent. We wanted to know exactly what was happening in the mitotic process during every minute between fertilization and cleavage. This necessitated preparation of sections. Eggs were fixed at one minute intervals either in Bouin's fluid or in Meves' fluid. We fixed four complete series of eggs, and all four series were imbedded in paraffin. Sections were made from only one series (Bouin), the others being kept in reserve. Sectioning and staining were done by Miss Drusilla Van Hoesen. Our sections are eight microns thick and they are stained with Heidenhain's hematoxylin.

## Results

The viscosity changes in the protoplasm of the Chaetopterus egg during the period between fertilization and cleavage are shown in Figure 1. The viscosity figures represent relative values and they have no absolute basis other than that they represent the number of seconds of centrifugation necessary to arrive at the endpoint described in the previous section. In order to obtain the viscosity values, after some preliminary tests, we ran through 12 complete series, in each of which the eggs were centrifuged at minute intervals for a given length of time. The times of centrifuging in the various tests were 4, 5, 7, 9, 10, 11, 12, 13, 14, 15, 17, and 18 seconds.

Fertilization is followed by a drop in viscosity; then while the maturation divisions are proceeding, the viscosity remains constant. In one or two of our series, we did get some indication that during the course of the maturation divisions, there

might be minor fluctuations in viscosity, but the weight of evidence is against any change whatsoever. This may seem strange, for in the egg of the clam Cumingia one of us had noted marked changes in viscosity during the maturation divisions (Heilbrunn, 1921). The difference between the egg of Chaetopterus and that of Cumingia is, however, easy to understand. In the relatively small egg of Cumingia, the maturation spindles occupy a rather large fraction of the egg volume. Thus Morris (1917) states that the first polar spindle "is large, and lies near the center of the egg. It might, in fact, be mistaken for a cleavage spindle in the metaphase, if it were not for the form of the chromosomes." Similarly, Jordan (1910) shows the first maturation spindle of Cumingia as a large structure extending through most of the egg; the distance between the centers of the centrospheres of this spindle is approximately half the diameter of the egg. On the other hand, the

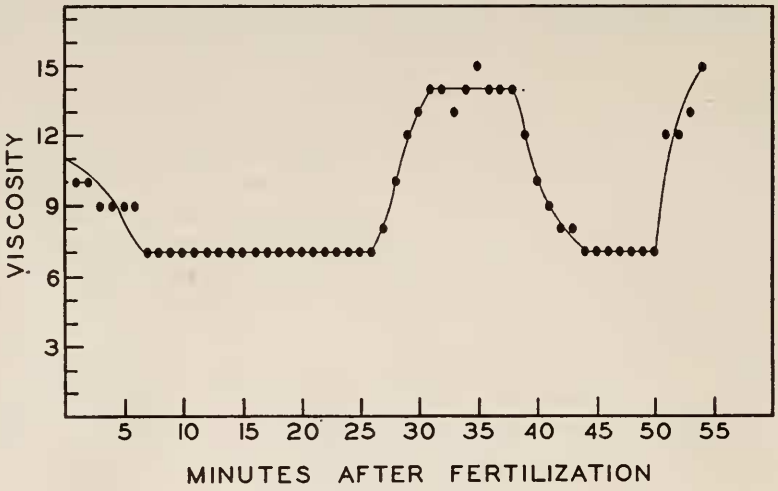


FIGURE 1. Protoplasmic viscosity changes in the egg of Chaetopterus during the time between fertilization and first cleavage.

maturation spindles of the Chaetopterus egg are relatively small. Thus, Lillie (1906) shows the fully formed maturation spindle of the Chaetopterus egg as small. In his Figures 3 and 4, the distance between the centers of its two centrospheres is only one-sixth of the diameter of the egg (Compare also Mead, 1898). Lillie (1906) showed that the cortex of the Chaetopterus egg was relatively viscous, containing granules which did not move when the egg was subjected to reasonably strong centrifugal force. We conceive, therefore, of the maturation spindles of the Chaetopterus egg as being relatively small bodies only several times as long as the cortical layer is thick and extending only a relatively short distance into the fluid region of the egg.

Following the maturation divisions, the protoplasm undergoes a sharp increase in viscosity. Our curve shows it to be approximately a two-fold increase. Some of our data indicated a somewhat greater change, but we preferred a conservative estimate. The viscosity increase is followed by a decrease in viscosity, and then

just before cleavage, the viscosity increases sharply again. These major changes in protoplasmic viscosity are related to the process of mitosis. In earlier work on the eggs of Cumingia and Arbacia, it was found that "the appearance of a spindle is preceded by an increase in viscosity and followed by a decrease in viscosity." It is of interest now to inquire as to whether the same correlations exist for the Chaetopterus egg.

Because of the fact that the Chaetopterus egg is one of the few invertebrate eggs that can conveniently be studied at Woods Hole, and because also of the present great interest in cell division, it was thought worth while to establish a complete time record of the mitotic changes in this egg as they occur during the interval between fertilization and first cleavage. One difficulty in presenting such a time record is the uncertainty of terminology. Mitosis is a continuous process and the various stages of this process can not be sharply delimited from each other. Moreover, not all authorities on mitosis agree in the way they define the stages. And even if a definition is rather uniformly followed, it is not always easy to apply it in such a way as to give a clear-cut decision as to when one stage ends and another begins. Thus, the telophase may be defined as the stage in which the chromosomes reach the poles of the spindle and begin to transform into vesicles or other structures characteristic of the resting stage. Now on a time basis, these two processes may not be simultaneous, and if one seeks to make sharp time distinctions, one must choose either the one or the other. Furthermore, the situation is complicated by the rapid succession of mitoses in an egg cell. Thus there may be no resting stage at all between two successive divisions, and there may not even be a complete telophase between the first and second maturation divisions.

For us it seemed wisest, if we were to present our results in tabular form, to make arbitrary criteria and distinctions. For our purposes, we shall define the metaphase as the stage in which the chromosomes are arranged along the equatorial plate of the spindle. We regard the anaphase as beginning as soon as the chromosomes have divided sufficiently so that we can see a space between the two groups of chromosomes. During anaphase, the two sets of chromosomes migrate toward the poles. It is hard to tell exactly when they have reached the poles. Accordingly, for this material, we chose as the distinction between anaphase and telophase, the moment when at least some of the chromosomes show signs of vesiculation. Actually, this distinction may depend to a slight extent on the depth to which the sections have been stained, but the difference between lightly and darkly stained sections does not appear to be great. On the basis of these distinctions, it was usually not too hard for us to tell when the egg cells were in metaphase, anaphase, or telophase. Prophases were more difficult. At the end of the first maturation division, after the first polar body has been separated off, there is an intermediate series of stages which are hard to classify. The polar body is given off 9 minutes after fertilization. At 10, 11, and 12 minutes after fertilization, one typically sees remnants of the first maturation spindle. At these times, the chromosomes are not vesiculated, so that according to our previous definition, it is not proper to call this stage a telophase. We might refer to it as an interphase, but we prefer to consider it as a late anaphase. At 13 minutes after fertilization, many half spindles appear in the sections. Following this is a stage in which the second maturation spindle appears; typically it lies in a plane perpendicular to the radius of the egg.

The chromosomes are frequently scattered along this second maturation spindle. This stage we refer to as the prophase of the second maturation spindle. The spindle then turns so that at metaphase it is perpendicular to the surface of the egg.

The second polar body is given off at 23 minutes after fertilization. The egg chromosomes now go into a very definite telophase stage and form discrete vacuoles. The egg nucleus is irregular in shape and may look like a bunch of grapes. At this stage, the male pronucleus, in sympathy as it were, may also become lobulated. There is thus a very definite telophase stage (at least in so far as the egg pronucleus is concerned). But between this telophase stage and the late prophase stage of the cleavage mitosis, it is hard to find distinctions which can be used for the purposes of our time scale. The male and the female pronuclei approach each other. There is thus a stage in which the pronuclei are separate and a stage when they are apposed. Usually, by the time they are apposed, the vesicular lobate appearance of the female pronucleus has disappeared so that this nucleus is now a smoothly spherical body with its chromatin either in a resting stage or in a condition indicative of an early prophase. There are exceptional cases in which the female pronucleus preserves its telophase appearance even though it is close to the male pronucleus. Then comes a stage in which the two pronuclei are fused together, or at least are apparently fused together. Mead (1898) says that an actual fusion of the pronuclei does not occur, but in many instances we were not able to detect a line of demarcation between the two pronuclei; and indeed in Mead's Figure 40, such a demarcation line is questionable. Accordingly, we distinguished a fusion nucleus stage. During this stage, the condition of the nucleus is almost certainly what most authors would call early prophase. There then comes a stage in which the nuclear membrane gradually breaks down and the chromosomes are arranged along the developing mitotic spindle; this stage is called late prophase.

In our time schedule, therefore, we distinguished the following stages: 1st maturation metaphase, 1st maturation anaphase, 2nd maturation prophase, 2nd maturation metaphase, 2nd maturation anaphase, 2nd maturation telophase, pronuclei separate, pronuclei apposed, fusion nucleus, late prophase of cleavage mitosis, metaphase, anaphase, and telophase (of cleavage mitosis). These stages are abbreviated in the headings of the table which gives a record of our findings. The data for the table were collected from a study of the slides prepared as described previously. For each minute of the time between fertilization and cleavage, we counted 20 cases in which the condition seemed clear in terms of one of the above-mentioned categories. The work was shared, and each of us made ten counts for each minute. On comparing our results, we found essential agreement.

The stages listed in our table do not give information on one point of considerable importance. They do not indicate at what moment the cleavage spindle first makes its appearance. After careful study of the sectioned material we have decided that the following series of events occurs. At 30 and 31 minutes after fertilization, the two pronuclei begin to come close to each other. There is at this time a large sperm aster with a large centrosphere. As the two pronuclei come still closer to each other (at 32 and 33 minutes after fertilization), between them they squeeze the centrosphere of the sperm aster into an elongated shape, so that it may form a narrow band between the two. This is the stage illustrated in Figure 39 of Mead (1898). This elongated centrosphere, with its astral rays

divided into two groups stretching well out into the cytoplasm, is not the definitive mitotic spindle, as subsequent stages indicate. Nevertheless, the line connecting the two sets of astral rays and the line along which the pronuclei fuse is almost always in the direction of the future spindle, for this line is typically perpendicular

TABLE I

*Mitotic stages in the Chaetopterus egg as a function of time (minutes) at 21° C. Further explanation in text*

| Time | 1st M | 1st A | 2nd P | 2nd M | 2nd A | 2nd T | PNS | PNA | FN | LP | M | A | T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 20 | | | | | | | | | | | | |
| 1 | 20 | | | | | | | | | | | | |
| 2 | 20 | | | | | | | | | | | | |
| 3 | 20 | | | | | | | | | | | | |
| 4 | 20 | | | | | | | | | | | | |
| 5 | 20 | | | | | | | | | | | | |
| 6 | 19 | 1 | | | | | | | | | | | |
| 7 | 12 | 8 | | | | | | | | | | | |
| 8 | 3 | 17 | | | | | | | | | | | |
| 9 | | 20* | | | | | | | | | | | |
| 10 | | 20 | | | | | | | | | | | |
| 11 | | 20 | | | | | | | | | | | |
| 12 | | 19 | 1 | | | | | | | | | | |
| 13 | | 15 | 5 | | | | | | | | | | |
| 14 | | 8 | 8 | 4 | | | | | | | | | |
| 15 | | | 9 | 11 | | | | | | | | | |
| 16 | | | 3 | 17 | | | | | | | | | |
| 17 | | | | 20 | | | | | | | | | |
| 18 | | | | 19 | 1 | | | | | | | | |
| 19 | | | | 16 | 4 | | | | | | | | |
| 20 | | | | 13 | 7 | | | | | | | | |
| 21 | | | | 6 | 14 | | | | | | | | |
| 22 | | | | 1 | 19 | | | | | | | | |
| 23 | | | | | 20* | | | | | | | | |
| 24 | | | | | 18 | 2 | | | | | | | |
| 25 | | | | | 8 | 12 | | | | | | | |
| 26 | | | | | | 20 | | | | | | | |
| 27 | | | | | | 18 | 2 | | | | | | |
| 28 | | | | | | 15 | 5 | | | | | | |
| 29 | | | | | | 14 | 4 | 2 | | | | | |

* Indicates time of appearance of 1st and 2nd polar bodies.

TABLE I—*Continued*

| Time | 1st M | 1st A | 2nd P | 2nd M | 2nd A | 2nd T | PNS | PNA | FN | LP | M | A | T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 30 | | | | | | 8 | 9 | 3 | | | | | |
| 31 | | | | | | 2 | 7 | 11 | | | | | |
| 32 | | | | | | 1 | 5 | 14 | | | | | |
| 33 | | | | | | | 1 | 19 | | | | | |
| 34 | | | | | | | | 20 | | | | | |
| 35 | | | | | | | | 20 | | | | | |
| 36 | | | | | | | | 9 | 11 | | | | |
| 37 | | | | | | | | 8 | 12 | | | | |
| 38 | | | | | | | | 3 | 17 | | | | |
| 39 | | | | | | | | 4 | 16 | | | | |
| 40 | | | | | | | | | 13 | 7 | | | |
| 41 | | | | | | | | | 5 | 15 | | | |
| 42 | | | | | | | | | 4 | 15 | 1 | | |
| 43 | | | | | | | | | | 16 | 4 | | |
| 44 | | | | | | | | | | 15 | 5 | | |
| 45 | | | | | | | | | | 3 | 17 | | |
| 46 | | | | | | | | | | | 19 | 1 | |
| 47 | | | | | | | | | | | 20 | | |
| 48 | | | | | | | | | | | 18 | 2 | |
| 49 | | | | | | | | | | | 16 | 4 | |
| 50 | | | | | | | | | | | 3 | 17 | |
| 51 | | | | | | | | | | | | 20 | |
| 52 | | | | | | | | | | | | 20 | |
| 53 | | | | | | | | | | | | 16 | 4 |
| 54 | | | | | | | | | | | | 6 | 14 |
| 55 | | | | | | | | | | | | 1 | 19 |
| 56 | | | | | | | | | | | | | 20 |

to the egg axis as indicated by the position of the polar bodies. This is also shown in Mead's Figure 39. Although at 32 and 33 minutes after fertilization, the astral rays are well developed, subsequently they seem to fade, so that 35 minutes after fertilization the asters either do not appear at all, or if present, they are faint. At this time there is no spindle. In the next three or four minutes, one occasionally sees instances of a double aster at one side of the fusion nucleus with what is apparently an embryonic spindle being stretched out between the two asters. Whether this is a general condition or not, only further study can decide. On one point we are certain, the definitive mitotic spindle does not appear until approximately 40 minutes after fertilization. In our study of eggs fixed at 40

minutes after fertilization, we found 15 out of 20 with the fusion nucleus elongated and pointed at its ends. In the pointed ends of these nuclei, spindle fibers show in 7 out of the 15 cases. Thus 7 out of 20 cells showed a true spindle. Probably this is somewhat less than the true proportion, for a nucleus might well be cut so that spindle fibers though present would not be visible. As far as our observations go, they indicate rather clearly that the moment at which the definitive mitotic spindle appears is 40 minutes after fertilization.

Let us now attempt to correlate our viscosity curve with the mitotic changes as shown by our observations of the fixed material. The viscosity curve shows a minimum of viscosity from 44 minutes after fertilization to 50 minutes after fertilization. This is almost exactly the time during which the cell is in metaphase, for the table shows the metaphase period to extend from 45 to 49 minutes after fertilization. We have chosen as the moment at which the definitive mitotic spindle appears as 40 minutes after fertilization. This is essentially simultaneous with the moment at which the viscosity of the protoplasm begins to drop.

We conclude therefore that the appearance of the cleavage spindle in the Chaetopterus egg is preceded by a period in which the protoplasm is relatively viscous. As soon as the spindle is formed, the viscosity drops. The metaphase is the stage at which the viscosity of the protoplasm is at a minimum.

Finally, we should like to express our admiration of the cytological study made by Mead in 1898. In general we confirm his findings. There are one or two minor points in which we might differ. In his Figure 46, which represents what we would call an anaphase, he shows some bodies in the equatorial plane of the spindle; these he calls nucleoli. Lillie (1906) in his Figure 25 illlustrates similar bodies which he labels as "chromatin masses cut off from the chromosomes." We have frequently seen these bodies in the equatorial plane of the spindle during the anaphase. However, our sections seem to indicate that they are neither nucleoli or chromosome fragments, but rather cytoplasmic granules which have pushed their way into the equatorial plane of the spindle. If this is correct, it is an observation which may have some importance in the interpretation of the mitotic spindle, but we made no careful study of the phenomenon. We should merely like to call the attention of the cytologists to it.

## Discussion

Our results provide a suitable basis for further work on the protoplasm of the dividing Chaetopterus egg, and we hope in the future to study the action of radiation and other agents in terms of their effect on the protoplasmic viscosity.

The viscosity curve that we have plotted for the Chaetopterus egg is essentially the same as that reported earlier for Arbacia and Cumingia. Heilbrunn (1921) stated that "The viscosity changes in Arbacia and Cumingia are absolutely parallel. In each case the appearance of a spindle is preceded by an increase in viscosity and followed by a decrease in viscosity. And in both eggs division of the cell is immediately preceded by a viscosity increase." As a matter of fact, all authors who have made objective measurements of protoplasmic viscosity during mitosis are in substantial agreement. A survey of much of the literature is given by Carlson (1946).

If one excludes the work done by subjective and non-quantitative methods, there is only one discordant paper. Fry and Parks in 1934 published what we believe to be a masterpiece of distortion. They made a few centrifuge measurements and then used Heilbrunn's data in plotting their curves, stating that Heilbrunn's measurements were more complete and accurate than their own. After doing this, they insist in a final discussion that Heilbrunn is wrong. They reach this strange conclusion by misquoting and distorting the views not only of Heilbrunn but also of almost every other worker in the field. Actually, though Fry and Parks claimed to have copied Heilbrunn's curves, this is not exactly true. The rise in viscosity which Heilbrunn found to occur in the Arbacia egg ten minutes after fertilization (for a cleavage time of 50 minutes) is shifted by Fry and Parks so that it occurs approximately seven or eight minutes after fertilization (for a cleavage time of 67 minutes). Thus in Heilbrunn's curve, viscosity rises only after one-fifth of the time between fertilization and cleavage has elapsed, whereas in the curve stated by Fry and Parks to be a copy of Heilbrunn's curve, the rise occurs when about one-ninth of the time between cleavage and fertilization has elapsed. Needless to say, this shift favors the interpretation Fry and Parks endorse. Moreover, the final upsweep of the Arbacia curve is shifted so as to make the metaphase of division come in a period of high rather than low viscosity. Fry and Parks claim to find agreement between their curves, which they state to be Heilbrunn's curves, and Chambers' opinions on viscosity change during mitosis, opinions based on subjective microdissection studies of various species of eggs at uncertain times. This they do in order to make Heilbrunn's curves fit what Fry and Parks regard as Chambers' opinions. In their Chart 5, Fry and Parks credit Chambers with maintaining that the metaphase is a stage during which the protoplasm is fluid. But this is the exact opposite of what Chambers says. Thus Chambers (1919) states: "The time of appearance of the amphiaster until completion of cleavage lasts from 10 to 15 minutes. The increased viscosity of the egg during the amphiaster stage could be more easily demonstrated by the needle in the eggs of Echinarachnius and Cerebratulus than in those of Arbacia."

The facts of the case are as we have stated them, and no amount of distortion can hide the fact that the appearance of the mitotic spindle is preceded by a stage of high viscosity and followed by a stage of low viscosity. Heilbrunn (1921) suggested that "it is as though the spindle were coagulated out of the protoplasm." Recent work has indicated that in some types of proteins, gelation may result in the formation of a spindle-shaped structure called a tactoid (Bernal and Fankuchen, 1941). Bernal (1940) believes that the spindle is actually a tactoid.

Perhaps there are other correlations that may be made between changes in the protoplasm and the series of viscosity changes that we have described. For one thing, the stage of increasing viscosity occurs at a time when water is being taken from the cytoplasm by the enlarging pronuclei. Then, when the spindle appears, the nuclear membrane breaks down and this might involve an increase in the water content of the cytoplasm. Carlson (1946) suggests that changes in the viscosity of protoplasm during mitosis may be due to changes in the nucleic acid content of the cytoplasm. He thinks that a high content of nucleic acid in the cytoplasm would tend to produce a high viscosity. Carlson states that Brachet and also Painter found the cytoplasmic nucleic acids abundant in early prophase, less abundant or entirely absent from late prophase through anaphase, and increasing in

amount following division; but in the papers cited by Carlson it is not possible for us to find any data on the changes in nucleic acid content of the cytoplasm during various stages of mitosis. That there is an exact correlation between the amount of cytoplasmic nucleic acid and the protoplasmic viscosity is very doubtful, and certainly it has not in any sense been established. For one thing, the unfertilized sea-urchin egg is apparently rich in cytoplasmic nucleic acids (see, for example, Caspersson and Schultz, 1940), and yet this unfertilized egg has a low protoplasmic viscosity (Heilbrunn, 1920).

## Summary

1. The viscosity of Chaetopterus egg protoplasm was determined at one minute intervals during the period between fertilization and cleavage.

2. By studying fixed, sectioned and stained material, the course of the mitotic processes in the Chaetopterus egg was followed minute by minute.

3. During the cleavage mitosis, marked changes in protoplasmic viscosity occur, and these are similar to the changes already described for the eggs of Arbacia and Cumingia.

4. The appearance of the mitotic spindle is preceded by an increase in protoplasmic viscosity and is followed by a decrease in protoplasmic viscosity.

5. During the metaphase, the protoplasmic viscosity is low.

6. Just before the cell divides, the protoplasmic viscosity increases markedly.

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